

The University of Chicago

STUDIES ON THE CONCENTRA-
TION AND LIBERATION OF
HISTAMINE IN TISSUES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1937

By

DAVID MINARD

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

1939

The University of Chicago

**STUDIES ON THE CONCENTRA-
TION AND LIBERATION OF
HISTAMINE IN TISSUES**

**A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF PHYSIOLOGY

1937

By

DAVID MINARD

**Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS**

1939



PREFACE

The study of pharmacologically active substances in tissue extracts has become so broad in recent years that it might almost be said to comprise a field in itself. Although histamine is but one of these substances, the investigations into its pharmacodynamic properties as well as its presence and distribution in body tissues had accumulated to such a degree that Feldberg (1930) was prompted to write a monograph covering the subject. During the past six years, the extension of our knowledge not only of histamine but of other vasodilator substances, some of which have come to light during that time, has called forth a new treatise which covers the more recent advances regarding the significance of these substances in physiological and pathological processes (Gaddum, 1936).

Before dealing with the special aspects of this field which have been selected for study, we might summarize very briefly the development of our knowledge regarding the properties of histamine and its significance.

As is true of other of these active constituents in tissue extracts, histamine was known chemically before its presence in nature or its pharmacodynamic properties were discovered, having been synthesized by Windaus and Vogt (1907).

Shortly thereafter Ackermann and Kutscher (1910) and Barger and Dale (1910) reported almost simultaneously its presence in nature as one of the constituents of the plant ergot.

Pharmacological studies followed (Dale and Laidlaw, 1910), and some of its more important properties including its depressor activity were brought to light. Hence the suggestion was made that histamine might be the hypothetical constituent in tissue extracts which Popielski (1909) had termed "vasodilatin."

Although Barger and Dale (1911) had isolated histamine chemically from the intestinal mucosa, this could not be considered as evidence of its presence in normal tissue, since contamination by the intestinal contents could not be ruled out. The evidence of its presence in tissues, therefore, still depended largely on

biological tests.

Further investigations into the pharmacological properties of histamine led to theories associating it with the phenomena occurring in anaphylactic shock (Dale, 1913, Dale and Laidlaw, 1919, etc.) and in traumatic shock (Cannon and Bayliss, 1919, and others).

That histamine or a substance closely similar to it was involved in the vascular reactions of the skin following injury became almost certain from the investigations of Lewis and his co-workers (1927).

The final proof that histamine was present in normal tissues was brought forward by Best, Dale, Dudley and Thorpe (1927) who succeeded in isolating histamine in chemical form from the liver and lung. By careful studies of the losses incurred during the isolation process, they were able to show that the quantities present were sufficient to account for the histamine-like activity of crude alcoholic extracts of these tissues. These facts added much greater significance to the findings in which biological tests had been used as the sole method of determination.

Studies of the changes undergone by histamine in tissues under various physiological and pathological conditions are complicated by the fact that histamine in some inactive form is a constituent of the normal tissue; in the process of extraction, the bonds holding the normally inactive histamine are broken. Hence to determine changes from the normal, changes which in themselves might be detectable, we must use as a basis of comparison the level of histamine already present in the normal tissue. Due to the relative insensitivity of our methods, the degree of change which might be expected to occur under experimental conditions may be too small to be appreciable.

However, in the face of these difficulties, we have selected as our problem the study of histamine in normal tissues and some of the factors which bring about changes in the normal level. Three phases of this problem have been studied, each phase more or less distinct in itself, and therefore each will be treated separately.

The author wishes to express his deep gratitude to Dr. Anton J. Carlson, whose constant guidance and encouragement made these investigations possible.

CONTENTS

	Page
PREFACE	11
Part	
I. THE EFFECT OF ULTRA-VIOLET IRRADIATION AND HEAT ON THE HISTAMINE-LIKE SUBSTANCES OF THE SKIN	1
Introduction	
Experimental Procedure	
Results	
Discussion	
II. THE PRESENCE AND DISTRIBUTION OF HISTAMINE IN BLOOD . .	12
Introduction	
Experimental Procedure	
Results	
Discussion	
III. HISTAMINE IN BLOOD FOLLOWING TRAUMA	27
Introduction	
Experimental Procedure	
Results	
Discussion	
IV. GENERAL SUMMARY AND CONCLUSIONS	34
LIST OF REFERENCES	36

PART I

THE EFFECT OF ULTRA-VIOLET IRRADIATION AND HEAT ON THE HISTAMINE-LIKE SUBSTANCES OF THE SKIN

Introduction

Although the presence of active vasodilator substances in extracts of normal tissues had long been recognized, suggestive evidence for their possessing a physiological role as local regulators of the small vessels was first brought forward by Ebbecke (1922), Krogh (1929), and by Thomas Lewis and his co-workers (1927). The latter author carried on an extensive series of investigations the results of which indicated that the cells of the epidermis contain a chemical substance in an inactive though easily dissociated form, which is liberated by various types of injury to the cells. The striking similarity between the typical "triple response" of the skin vessels to injurious agents and the vascular reactions to small quantities of histamine pricked into the skin led Lewis to believe that the theoretical substance or substances involved are closely related to histamine if not histamine itself.

Suggestive though the work of Thomas Lewis was, direct evidence for changes in the histamine content of the skin following injury was lacking. In an effort to supply such evidence, Harris (1927), working in collaboration with Lewis, undertook the study of histamine in normal skin and in skin which had been burned or frozen. Using the cat blood pressure and the guinea pig uterus as biological test objects for the quantitative assay of histamine-like substances in alcoholic extracts of the skin of the cat, Harris observed that skin removed immediately after being burned or frozen possessed no more nor less histamine than normal skin. However, if allowed to remain in situ for an hour following injury, the skin was shown to possess only about 50 per cent of its original histamine content. Harris offers the explanation that damage to the cells allows the preformed histamine to diffuse out of the cells and into the tissue spaces where it is removed by the blood and lymph.

The discovery that ultra-violet irradiation of histidine solutions leads to the formation of small quantities of histamine prompted Ellinger (1929) to study the effect of such irradiation on the histamine content of skin. Skin areas in guinea pigs were irradiated with a mercury-vapor lamp, and at varying intervals equal portions of irradiated and non-irradiated areas were removed, extracted with hot chloroform, the final aqueous extracts being tested on intestinal strips of the guinea pig and blood pressure of the cat. In three of the ten cases studied there was an increase of histamine-like substance in the irradiated area, in two cases questionable results were obtained, and in the other five animals no difference between irradiated and non-irradiated tissues was observed. Ellinger suggested the theory that minute quantities of histamine, formed by the irradiation in the superficial layers of the epidermis, diffuse slowly to the deeper tissues, there causing the vascular reactions.

Diehl (1931) demonstrated that strong irradiation with rapidly appearing erythema causes the appearance of free HCl in the gastric juice of histamine-positive achylia patients; hence he concludes that a substance is liberated possessing a histamine-like action.

Using a phosphotungstic-acid precipitation procedure to partially purify his extracts, Loos (1931) observed up to eight times the quantity of intestinally active substances in extracts of burned rabbit skin as compared to extracts of normal skin. He ascribed the increase to the leucocytic migration into the injured area.

Two preliminary reports of work bearing on this problem have recently appeared. Using iontophoresis Harpuder (1935) was able to recover from areas of skin treated with ultra-violet irradiation, diathermy, and the radiant lamp substances which cause an increase in tonus of the isolated guinea pig intestine, the action not being counteracted by atropin. These substances were not obtained from normal skin.

Bartosch (1936) within the last year has reported the appearance of substances possessing many of the pharmacological and physicochemical properties of histamine in the perfusion fluid of guinea pig lungs following the application of irritating chemical substances. The isolated rabbit ear gave analogous results and in addition the application of hot water caused the

previously inactive perfusion fluid to acquire histamine-like properties.

The purpose of the following investigation was to determine whether the normal skin content of histamine-like substances can be altered by ultra-violet irradiation and heat, and if so, whether both factors cause a change in the same direction.

Experimental Procedure

The tissue selected for this study was the skin of albino rabbits, the ears of this species offering relatively large, flat unpigmented skin surfaces suitable for uniform irradiation as well as facilitating the removal of equal areas of irradiated and non-irradiated regions.

Irradiation.--The source of ultra-violet irradiation was a carbon-arc lamp employing Eveready "C" carbons and operated on an A.C. 110v-25A line. In thirteen animals the skin of the experimental ear was irradiated with the naked arc while in the remaining four animals a Corex A No. 986 filter was interposed between the arc and tissue.

Preliminary experiments were carried out to determine the distance of the ear from the source and the duration of exposure most suitable for the production of a marked erythematic response without blister formation. These were found to be 70 cm. without filter and 35 cm. with filter, the duration of exposure being twenty minutes in both cases.

Prior to irradiation both ears were carefully clipped free of hair, shaving having been found to cause irritation to the skin.

Irradiation was carried out with the animal in a rabbit box of conventional design with the addition of a movable asbestos screen which served at once as a support for the ear to be irradiated and as a shield for the control ear and head of the animal. In some experiments using the naked arc, the control ear was simultaneously irradiated with the experimental ear but through a pane of ordinary window glass 3 mm. thick. No erythema in the control ear was observed after such treatment and no difference in the final results was obtained.

The typical ultra-violet erythema, accompanied by oedema, was first noticed between three to six hours following irradiation, during the latent period both ears appearing normal. This

erythema tended to increase in intensity up to thirty-six hours after irradiation, and between forty-eight to seventy-two hours the erythema and oedema subsided, the skin taking on a yellowish tint, the final stage being a desquamation of the dead layer of epidermis.

At varying intervals following irradiation the ears were amputated, either with scissors immediately after killing the animal with a blow on the head, or with an electro-cautery knife, the animal being under ether anaesthesia.

The removed portion of the two ears were quickly cut down to the size of a rhomboidal cardboard pattern 22 cm² in area. In seven rabbits each skin surface was removed from the cartilage, weighed separately and placed in a beaker containing approximately ten times the tissue volume of 95 per cent alcohol. In ten experiments the ears were cut down to the same sized pattern as above but the two skin surfaces were left attached to the cartilage and the three layers of tissue extracted together. After hardening the tissue was finely divided in the beaker with scissors, the minced tissue and alcohol being then transferred to an Erlenmeyer flask. After twenty-four hours the primary alcoholic extract was filtered off and the tissue re-extracted another twenty-four hours with 70 per cent alcohol. The primary and secondary extracts were then combined, evaporated to dryness on a water bath at 40°C., the residue taken up in Ringer solution, washed with ether in a separatory funnel to remove lipoids, and finally made up to a volume of 25 cc. in cases where both surfaces of skin were extracted or 12.5 cc. when one surface alone was extracted.

Heat.--In the three experiments involving the effect of heat, the experimental ear was immersed in water at 54°C. for four minutes. In one experiment the treated and non-treated areas were removed after thirty minutes. In the other two experiments an interval of three hours elapsed between burning and removal. The procedure of removal and extraction was identical to that described above.

Quantitative assays of the histamine-like activity of the extracts were carried out on the etherized vagotomized cat, the blood pressure being recorded in the usual manner by a mercury manometer attached to the cannulated carotid artery. The test solutions were injected into the cannulated femoral vein and washed through with a constant amount of warm saline.

In agreement with Harris' findings early experiments showed atropin to have no effect on the assay; hence atropinization was dispensed with in the later experiments.

The standard of comparison used in all these experiments was a 3.0×10^{-6} solution of histamine acid phosphate. The extracts of the experimental and control skin were first assayed against one another to obtain the relative difference of activity and then either or both were assayed against the histamine standard to obtain an estimate of the absolute concentration of histamine-like activity in the extract. The results were expressed in terms of gamma equivalents of histamine base both per gram of tissue and per cm^2 skin surface. In many experiments a minimal change of 15 per cent in the dosage could be detected. In some experiments the smallest appreciable difference in dose was 20 per cent.

Rabbit blood pressure.--As a qualitative test for the absence of depressor substances other than histamine the effect of some extracts was tested on the blood pressure of the rabbit under ether anaesthesia, Major and Weber (1929) having shown that depressor substances present in tissue extracts which act like histamine in other ways always cause a fall in pressure in the rabbit. Small quantities of histamine (.25, -10 γ) have no effect on the rabbit blood pressure under ether anaesthesia while larger doses cause a slight rise.

Blood samples.--In six experiments approximately 4 cc. samples of venous blood were taken from the lateral vein of the treated and non-treated ears, after venous drainage had been occluded, the blood being allowed to drop directly into weighed tubes containing the precipitating agent, which was five times the volume of alcohol in two experiments and 7.5 cc. of 10 per cent trichloroacetic acid in the other four experiments. The alcoholic extraction procedure was identical with that used in making the skin extracts while the trichloroacetic acid extraction procedure was that described by Barsoum and Gaddum (1935a). Assays were made using the cat blood pressure.

Pauly reaction.--With Hanke and Koessler's (1920) modification of the Pauly reaction qualitative comparisons for iminazoles were performed on extracts from eight of the irradiation experiments and the three experiments on burns. Since difficulty was encountered in obtaining a color standard to match the color of the test solutions and in view of the fact that comparative

rather than absolute values were desired, the relative intensities of the color reactions were obtained by using equal quantities of the two extracts in each cup of the colorimeter, and the height of one cup adjusted until the colors matched.

Norit adsorption.--Histamine is completely adsorbed by Norit while some other unidentified depressor substances, found particularly in brain but in other tissues as well, are not adsorbed. Ten cc. of the skin extract were shaken with 2 grams of Norit for ten minutes and filtered. The filtrate was tested for depressor activity using a portion of the untreated extract as a control.

Results

The results of the experiments on irradiation and burning are given in detail in the accompanying tables.

Explanation is required in several instances. In the case of animal 12, both ears were subjected to irradiation; the data listed under column headed "Irradiation" is for the ear irradiated twenty-four hours before removal and under the "control" column is for the ear irradiated four hours before removal. The ear irradiated four hours before removal showed some signs of erythema.

Animals 1-13 were irradiated with the naked arc, while animals 17-20 were irradiated through the Corex A 986 filter.

Animals 19 and 20 both underwent irradiation six hours before the ears were amputated. However, no signs of erythema or oedema of the irradiated ear were evident in case of animal 19, while animal 20 showed a marked response at this time.

In animal 1 the ears were removed and cut to the same size but the area was not determined.

Irradiation.--In every case except rabbit 8 the irradiated tissue showed a decrease in the histamine equivalent (H.E.) per gram of tissue due to the oedematous condition of the skin at the height of the response to irradiation.

On the other hand the total H.E. of the irradiated tissue as compared to the control was found to be increased in nineteen of the twenty-two cases studied, the average increase being 38 per cent (range 0-100).

Of the three negative cases, animal 19, in which the ears were removed six hours after irradiation, displayed no signs of

TABLE I
EFFECT OF IRRADIATION

Rabbit Number	Interval between Irradiation and Removal	Surface of Ear from Which Skin Removed	Irradiated			Control			Difference between Total H.E. Irradiated and H.E. Control
			Weight of Tissue	Histamine Equivalent	Histamine Equivalent	Weight of Tissue	Histamine Equivalent	Histamine Equivalent	
	hours		grams	γ per gram	γ per cm^2	grams	γ per gram	γ per cm^2	$\%$
7	24	Outer	1.08	10	.45	.68	14	.45	0
		Inner	.67	7.4	.22	.40	9.3	.17	+35
8	24	Outer	1.11	13	.68	.74	14	.47	+45
		Inner	.64	15	.45	.41	12	.22	+100
9	24	Outer	1.44	10	.68	.51	24	.53	+25
		Inner	.61	14	.38	.39	16	.26	+45
10	24	Outer	1.36	12	.80	.65	21	.62	+30
		Inner	.91	12	.51	.51	17	.40	+30
11	24	Outer	2.20	10	1.0	.83	21	.80	+30
		Inner	1.02	11	.51	.59	14	.40	+30
13	24	Outer	1.65	10	.80	.84	14	.56	+40
		Inner	.81	10	.40	.51	12	.28	+40
2	24	Outer							
		Inner and Cartilage	5.23	5.8	.70	2.60	9.3	.55	+30
3	24	"	4.30	5.2	.50	1.93	8.1	.35	+40
4	24	"	3.42	4.0	.27	1.61	7.8	.27	0
5	24	"	5.06	4.0	.45	1.93	7.7	.34	+35
6	24	"	4.59	4.3	.45	2.02	7.5	.34	+35
17	24	"	4.16	10	.90	1.94	13	.55	+70
18	12	"	3.59	11	.90	2.00	12	.55	+60
19	6	"	2.18	9.0	.45	2.06	10	.45	0
20	6	"	2.33	8.7	.45	1.71	8.8	.35	+30
1	68	"	5.60	7.1	--	2.51	8.0	--	+100
12	24-4	Outer	2.84	4.4	.56	1.54	8.1	.56	0
		Inner	1.14	6.5	.34	.79	7.9	.28	+20

TABLE II
EFFECT OF HEAT

Rabbit Number	Interval between Burning and Removal	Surface of Ear from Which Skin Removed	Burned			Control			Difference between Total H.E. Burned and H.E. Control
			Weight of Tissue	Histamine Equivalent	Histamine Equivalent	Weight of Tissue	Histamine Equivalent	Histamine Equivalent	
			grams	γ per gram	γ per cm^2	grams	γ per gram	γ per cm^2	
14	30 min.	Outer	1.65	7.5	.56	.58	21	.56	0
		Inner	.88	5.6	.22	.48	10	.22	0
15	3 hrs.	Outer							
		Inner and Cartilage	6.64	4.9	.73	2.10	19	.90	-20
16	3 hrs.	"	4.35	6.6	.62	2.56	13	.78	-20

erythema at that time.

In the second case, animal 6, only one surface of the irradiated ear showed no increase. The total H.E. for both surfaces was increased as compared with the total H.E. of the control.

In no case was the total H.E. of the irradiated tissue less than that of the control.

Statistical treatment of the data tends to rule out the factor of chance variation:

$$\text{Diff} = 2.82 \text{ Standard error}$$

This indicates a high probability that the changes observed are significant.

Heat.--The results obtained in the three experiments in each of which one ear was burned for four minutes at 54°C. are given in Table II.

In animal 14 the burned ear and its control were removed thirty minutes after treatment, the inner and outer surface of each ear being extracted separately. No difference in the total H.E. of the burned and normal tissue was detectable.

In two experiments the burned ear and the control were removed three hours following treatment. The total H.E. of the burned tissues was found to be 20 per cent less than normal in each case.

It was observed that the degree of oedema closely approximated or exceeded that seen in the irradiated ears.

Rabbit blood pressure.--Extracts of irradiated and normal skin gave no depressor response in the rabbit under deep ether anaesthesia.

Blood samples.--Extracts of venous blood drawn from the normal and irradiated ears and extracted either with alcohol or trichloroacetic acid showed no consistent difference when tested for histamine-like activity on the blood pressure of the cat.

Pauly reaction.--Studies using Hanke and Koessler's modification of the Pauly reaction revealed a 10-40 per cent more intense color from crude extracts of irradiated skin as compared with similar extracts of control areas. Further purification of the extracts with amyl alcohol was not carried out.

In the first experiment on burned tissue, no difference was observed in the intensity of the color reaction of the extracts of the burned tissue when compared with the controls. In the other two experiments, extracts of the burned skin yielded approximately 10 per cent more intense color than did the controls.

Norit adsorption.--Extracts of skin treated with Norit were completely inactive in producing a depressor response in the cat.

Discussion

In considering these data, the question at once arises as to the origin of the added histamine-like substances in the irradiated tissue.

The most obvious explanation which comes to mind is that some constituent of the blood plasma entering the injured area is responsible for the increase. However, in view of the fact that an equivalent degree of oedema was present in both irradiated and burned tissues and since each of these displayed a diametrically opposite trend regarding the substances concerned, such an explanation seems hardly tenable. Unless we postulate a qualitative difference in the constitution of the plasma transudate in the two types of injury, we must conclude that the origin of the added

histamine is from the tissue cells themselves. Further evidence for this view is that plasma of normal rabbit blood contains relatively minute quantities of the histamine-like substance. Reports of investigations on histamine in blood will be made later.

However, it may also be seen from the data that the total increase in H.E. following irradiation is coincident with, but not proportional to, the increase in extra-vascular fluid as measured by the increase in weight of the tissue. To have found such a direct proportionality would not have been surprising, as it would have suggested the existence of a causative relationship. The absence of the relationship adds support to the view that the origin of the added histamine is from the tissue but, on the other hand, considerably weakens the evidence that the increased quantity of histamine present in the tissue following irradiation is one of the main factors in producing the local vascular responses.

In general the results of these experiments add direct support to those of Harris, who found a decreased quantity of histamine following burns, and differ only in degree from Ellinger's findings with irradiation, the latter's results being largely negative. Since these two investigators obtained results which were contradictory, in view of Lewis' work, the discrepancy might have been explained as being due either to a species variation, the one having used cats, the other guinea pigs, or to the different methods of extraction and assay employed. The findings presented here were obtained using a constant set of experimental conditions; the only factor varied was the type of injury imposed on the tissue. Hence it seems safe to conclude that different injurious agents may have opposite effects on the normal histamine content of the skin.

Although the results with irradiation presented here do not conflict with those of Ellinger, nevertheless they do not support his theory as to the mechanism involved, namely that during exposure to the U.V. irradiation small quantities of histamine are formed in the superficial layers of the epidermis and subsequently diffuse slowly to the deeper layers of tissue, there producing the vascular reactions. If this were so, one would expect the increase in histamine level of the skin to be immediate and to diminish progressively as erythema developed. Such does

not seem to be the case. On the contrary, in our experience, the increase was not observed until erythema had already appeared.

Neither could we find support for Lewis' theory that U.V. irradiation, like all other forms of injury simply liberates the preformed H-substance from the cells.

A theory which might explain some of the differences between the effect of irradiation and heat on the histamine content of the skin, as well as accounting for the latent period between irradiation and the appearance of the erythema, is the following:

Small quantities of histamine are continually being formed by the cells of the epidermis but the concentration is normally prevented from reaching a sufficiently high level to produce vascular effects by some mechanism, perhaps enzymatic in nature, which destroys the histamine either at the site of formation or immediately after diffusing from the cells. The short wave irradiation has a destructive influence on this mechanism thus permitting histamine to accumulate slowly until it reaches the critical level of concentration to bring about the vascular effects. Burning and perhaps other types of injury, in accordance with Lewis' view, liberate preformed histamine from the damaged cells, the response being, therefore, immediate.

Although this paper has dealt exclusively with depressor substances in the skin which resemble histamine, it must be emphasized that despite the correlation between changes occurring in the H.E. (histamine equivalent content) of the skin following injury by burns and irradiation and the vascular responses to such injury, there is as yet no evidence that the substances studied are identical with histamine nor that the altered H.E. is in any way directly responsible for the vascular reactions. Indeed it has recently been suggested rather strongly by Menkin (1936) that chemical substances other than histamine probably play a more important role in local inflammatory changes.

PART II

THE PRESENCE AND DISTRIBUTION OF HISTAMINE IN BLOOD

Introduction

Until recently efforts by various investigators to identify histamine in normal blood have failed (Hanke and Koessler, 1924; Guttentag, 1931; Zipf and Hulsmeyer, 1933; MacGregor and Thorpe, 1933). Although Harris (1927) considered that pharmacological tests on alcoholic extracts of blood and blister fluid indicated the presence of histamine, it now seems likely that he was dealing with more than one biologically active substance. More recently Page (1935) observed that extracts of rabbit blood possessed more depressor activity than blood of other species; it is probable that the difference was due to the greater histamine content of rabbit blood.

Barsoum and Gaddum (1935a) have recently reported positive evidence of the presence of histamine in normal blood from a number of animal species. Boiling extracts of blood with hydrochloric acid was found to destroy other pharmacologically active substances, and the remaining activity was dependent solely on the presence of histamine, which is stable to such treatment. This fact was established by employing as the assay object the isolated rectal coecum of the fowl which is readily desensitized to histamine while retaining its responsiveness to other intestinally active substances. Acid treated extracts, tested on the atropinized coecum before and after histamine desensitization, were shown to contain only histamine.

These authors reported a remarkable species variation in histamine content of blood, the extremes being 10γ (gamma) per cc. for the rabbit and 10γ (gamma) per liter for the cat. The distribution of histamine between corpuscles and plasma was first stated to be in the ratio of 10:1 for rabbit blood. Later Anrep and Barsoum (1935a) reported the RBC:Plasma ratio of histamine may be as high as 18:1 in the rabbit but 1:1 for the dog.

In reporting the presence of histamine in dialysates of

rabbit blood, Tarras-Wahlberg (1936) distinguished between a "free" and "bound" phase in the plasma histamine, the former presumably being in a biologically active form, the latter in some inactive combination with a plasma constituent.

At the time the present investigations were begun the primary purpose in mind was to develop better quantitative methods for the extraction and assay of histamine suitable for the detection of small changes in blood histamine concentration which might be expected to occur in blood leaving an injured area. Secondly, it was decided to reinvestigate the distribution of histamine in blood to determine to what extent transudation of plasma into injured areas might be held responsible for changes occurring in the histamine content of injured tissues, since it had previously been observed that following irradiation of the skin there was an increase in the total histamine content of the skin after erythema and oedema had developed.

It was only when certain discrepancies in the histamine equivalent of blood plasma in different experiments prompted a more thorough search for the true site of histamine in blood that the findings which form the principal basis of this paper came to light.

Experimental Procedure

Two species of animals, the rabbit and the dog, served as donors of the blood used in these experiments.

Collection of blood samples.--Rabbit: Samples were obtained from the lateral vein of the ear, which had previously been shaved, made hyperemic by heating with an electric bulb, and coated with vaseline. The vein having been nicked with a razor, the blood was allowed to drop directly into the anticoagulant contained in a 15 cc. graduated centrifuge tube. In the majority of the earlier experiments .07 cc of 5 per cent sodium citrate per cc. of blood was used and made up to a volume equal to that of the blood to be obtained with calcium-free Ringers solution. In later experiments, when platelet volumes were to be measured, 1.3 per cent sodium oxalate served as the anticoagulant. In a few experiments on undiluted blood heparin was employed.

Extreme precautions were taken in preventing coagulation of the blood. Drops falling at a rate of less than two per

second were not collected. If during collection of a sample a drop fell onto the side of the vessel and not directly into the anticoagulant, such samples were discarded. The tubes containing the anticoagulant were chilled in an ice bath and immediately replaced after collection of the sample.

All samples of plasma used in the experiments were prepared by centrifuging the blood for twenty minutes in a refrigerator room. In later experiments to avoid contamination of the samples with platelets, the plasma was removed from the precipitated cells and recentrifuged another twenty minutes.

In the later studies, platelet-rich, cell-poor suspensions were obtained by centrifuging 10 cc. of the diluted blood (1:1) for one and one-half to two minutes at 2,300 r.p.m. Quantitative platelet volume methods showed such suspensions to contain from 50-85 per cent of the platelet volume of the original diluted blood, while over 99 per cent of the red cells, and 95-100 per cent of the white cells were absent.

Platelet volume studies on normal blood were performed using the thrombocytocrit technique of Van Allen (1926), modified to fit the conditions of the experiment. Nine cc. of blood were collected as above into 6 cc. of 1.3 per cent sodium oxalate and thoroughly mixed by drawing the blood into a pipette and gently expelling, this being repeated several times. This sample was then divided into three 5 cc. portions each of which was diluted to 15 cc. with 1.3 per cent sodium oxalate and mixed again. Hence in this final dilution 1 cc. of the original blood had been made to 5 cc. with the oxalate solution. Two 10 cc. portions of this diluted blood were placed in round-bottom sedimentation flasks and allowed to remain undisturbed for three and one-half hours at room temperature. The remainder of the procedure is identical with that described by Van Allen.

The platelet concentration in the centrifuged specimens was determined by diluting that quantity of suspension obtained from 1 cc. of the original blood to 5 cc. with the oxalate solution. This made possible direct comparison of the relative platelet volumes in the suspension and in the original blood.

Dog: In the studies on dog blood, samples were obtained by venapuncture, the syringe containing .5 cc. 5 per cent sodium citrate for 10 cc. of blood. Platelet suspensions were obtained as above except that larger quantities of blood were used.

Thirty cc. of blood were drawn into a syringe containing 30 cc. of 1.3 per cent sodium oxalate, mixed and centrifuged three minutes. The pink, cloudy plasma was then removed from the cells and recentrifuged for three minutes to throw down most of the remaining corpuscular elements. A 2 cc. sample of this suspension was taken for a thrombocytae determination and 50 cc. of that remaining was centrifuged for one hour. The clear plasma was decanted off and the precipitated mass of platelets stirred up in 10 cc. of calcium-free Ringers and extracted in parallel with the whole citrate blood sample.

Hematocrit determinations were obtained on all samples used.

Methods of extraction.--Electrodialysis: The method of electrodialysis described by MacGregor and Thorpe (1933) was employed in the majority of these experiments. However, since certain modifications in apparatus and procedure have been introduced, the method used here will be described in detail:

Three-chambered cells were used, each cell consisting of two electrodal chambers cut out of solid blocks of wood and impregnated thoroughly with paraffin. The middle chamber was made from pure gum rubber, thus eliminating the necessity of the four rubber gaskets. With the electrode in place each chamber was 11 cm. deep, 5 cm. wide, and 1 cm. thick. A nickel cathode and carbon anode, each of the same area as the face of the chamber, were fitted snugly against the wall of the corresponding chamber, and sealed in place with paraffin. This reduced the number of movable parts and insured a more thorough washing of the chamber. Glass cooling coils were used only in the electrodal chambers. Membranes of heavy parchment paper separated the three chambers.

Four such cells were connected in parallel with a 110 volt D.C. line, a bank of three 75 watt bulbs in parallel being in series with the cells. The bulbs were successively placed in the circuit and at the end of half an hour were shunted out entirely.

Twenty-five cc. of distilled water were used in each of the electrodal chambers, and 20 cc. in the middle chamber. The blood samples to be dialyzed were placed in the middle chambers of the cells, care being taken that the same equivalent quantity of electrolyte was added to each cell. The smallest samples used were .5 cc. blood in 2.5 cc. of isotonic electrolyte and the

largest samples, 5 cc. of heparinized whole blood.

As a precautionary measure, dialysis was continued for an hour in all cases, although preliminary experiments indicated that the process was probably completed in about half that time. The colorimetric method used by MacGregor and Thorpe to determine the end point of the dialysis was not used. This seems justifiable since we are dealing here with the same material in every instance.

At the end of an hour, the liquid from each cathodal chamber was removed, the chambers washed out three times, and the washings added to the cathodal liquids, which were then neutralized with .2 NHC1 against phenolphthalein, and made to 50 cc. with dist H₂O. The dialysates were then ready for assay or further treatment.

To determine the stability of the substance in the dialysate to boiling in acid, or in order to concentrate the activity, 5 cc. of NHC1 was added to each 15 cc. of the dialysate which was then boiled on a sand bath for one and one-half hours, evaporated to dryness in vacuo on a boiling water bath, the residue washed twice with 15 cc. of 95 per cent alcohol, each time the alcohol being driven off under reduced pressure, and the final residue taken up in water, neutralized with .2 NNaOH and made up to the required volume.

Trichloroacetic acid extraction: In order to check the electro-dialytic method against the trichloroacetic acid extraction method of Barsoun and Gaddum (1935a), several experiments on rabbits' blood were carried out using the procedure as described by these authors. Samples were collected as above but in this case the blood was allowed to drop directly into weighed tubes containing the 10 per cent trichloroacetic acid. Samples for dialysis were obtained at the same time.

In the dog experiments, trichloroacetic acid extraction was used exclusively, since the relatively low histamine concentration in the blood of this species required a quantity of blood in excess of that which the electro-dialytic cells could adequately handle.

Methods of assay.--Isolated guinea-pig intestine: The isolated intestinal strip of the guinea pig has been used by many investigators since the time of Guggenheim and Loeffler (1916) as a convenient assay object for histamine.

However, the degree of sensitivity achieved with this

preparation seems to vary considerably in the hands of different investigators. For example, according to Ellinger (1929), the sensitivity of the guinea pig strip will only differentiate between concentrations ranging $\frac{1}{100}$, $\frac{1}{300}$, $\frac{1}{500}$, $\frac{1}{1000}$ mgm. Guttentag (1931), too, was able to obtain results with an accuracy of only ± 15 per cent. On the other hand Barsoum and Gaddum (1935a) report the preparation to be sensitive to changes of ± 5 per cent, which so far as our knowledge goes, is the maximum sensitivity reported in the literature.

In so far as we have, by modifying the usual procedure to some extent, consistently obtained results accurate to ± 5 per cent, and on frequent occasions have achieved an accuracy of ± 2.5 per cent, it would, perhaps, be within the scope of this paper to present an account of the method as employed by us.

The tissue bath and heater used were essentially the same as described by Burn (1928) with the exception that the Ringer-Locke solution was led through a glass coil inside the heating chamber before entering the bath, which had a capacity of 25 cc.

The Ringer-Locke solution was in all cases freshly prepared before each experiment from water redistilled in glass and from concentrated stock solutions kept in an ice-box. The concentration of the constituents of the Ringer-Locke solution were: NaCl - .92%; KCl - .042%; CaCl_2 - .025%; NaHCO_3 - .015%; dextrose .1%.

After starving for forty-eight hours, the guinea pig was killed by a blow on the head, the abdomen quickly opened, and a portion of the empty ileum, 10-20 cm. long, removed placed immediately in chilled aerated Ringer-Locke solution, and set in an ice-box. If the Ringer-Locke solution was changed every forty-eight hours such preparations could be kept in good condition up to ten days, successful assays having been performed on strips preserved for that length of time. As a rule much more regular and uniform responses were obtained from strips three to seven days old than with the fresher preparations. The sensitivity and magnitude of response were not significantly diminished up to a week after removal.

Segments of gut, 2-3 cm. long, were removed from the ice-box, the mesentery cut away, and the strip suspended in the aerated bath maintained at 37°C . The frontal writing lever of aluminum was mounted on jeweled bearings and tipped with a

delicately balanced glass capillary. The ratio of the lever arms was 15:1. Records were made on a lightly-smoked drum surface which prior to the experiment had been ruled with parallel lines $\frac{1}{8}$ cm. apart.

During the first twenty-thirty minutes in the bath, preserved strips showed no activity whatever, and would respond very weakly if at all to relatively large quantities of histamine. Gradually recovery would occur, and increasingly large responses to the same dose of histamine were obtained, until a constant level was reached.

In all the later experiments the following modification in the usual procedure was followed: The bath was washed out twice with the standard Ringer-Locke solution after each test. Shortly thereafter 1 cc. of .5 per cent CaCl_2 was added to the bath. This immediately caused a complete relaxation of the strip, followed in thirty-sixty seconds by some return of the spontaneous motility; two to three minutes after the last test solution had been added the strip was again ready for testing. The bath having been washed out and refilled with the normal Ringer-Locke, the strip would contract. This contraction was immediately counteracted by the calcium added after each washing.

In our experience, this procedure has consistently yielded good results. Even those strips preserved for a week or more in the ice-box have sometimes given remarkably uniform and quantitative responses to histamine added at two or three minute intervals for the course of several hours.

The beneficial effect of the added calcium, as well as the preservation of the strip at ice-box temperature, seems to be primarily a stabilization of the activity of the strip by eliminating or controlling spontaneous changes in tone and pendular movements, and by maintaining a constant set of conditions prior to each test.

In these experiments, a solution of histamine di-phosphate equivalent to a $.1 \times 10^{-6}$ solution of the basic amine was used as the standard of comparison. This had been found to be closely equivalent to the concentration of the histamine-like activity in the electrolysates as prepared above. As a rule experimental samples were first assayed against one another to obtain the relative degree of activity in each, and then one or more samples were assayed against the histamine standard to obtain an estimate

of the absolute concentration of histamine in each. Only after successive checks with varying doses was the assay of any sample said to be complete.

All assays have been within the limits of accuracy of ± 5 per cent. In some experiments we feel justified in reporting an accuracy of ± 2.5 per cent.

Blood pressure of the etherized cat: In a few experiments samples were compared with a histamine standard (1.0×10^{-6}) on the blood pressure of the etherized cat before as well as after the administration of atropin. The carotid blood pressure was recorded with a mercury manometer and test solutions were injected into the cannulated femoral vein.

Results

Rabbit.--Whole blood: Quantitative estimations of histamine equivalents in electrodialysates of blood from several rabbits have given a range of values varying from 2.8×10^{-6} to 5.0×10^{-6} of the basic amine for the original blood. Samples from the same rabbit taken at intervals of several days showed a more narrow range of variation.

(1) In a series of control studies twelve samples were dialyzed in duplicate, six samples in triplicate, and four samples in quadruplicate. In one instance the histamine equivalent of a sample differed from that of its control by about 10 per cent. In all other cases agreement was within the limits of accuracy of the assay. Redialysis of the middle chamber liquid for one hour and concentration of the resulting cathodal liquid revealed the complete absence of intestinally active substances.

(2) More than 90 per cent of 5 gamma of histamine added either alone to the middle chamber or to the blood sample to be dialyzed could be recovered (four experiments). In two experiments complete recovery was observed.

(3) In ten experiments a portion of the blood dialysate, treated with acid and boiling as described above, was compared in activity with the non-treated portion. In six cases there was no appreciable difference; in two cases, an increase of 10 per cent; in one case an increase of 20 per cent; and in one case a 10 per cent decrease.

(4) Close quantitative agreement was observed between

electrodialysates and trichloroacetic acid extracts of duplicate samples. In three experiments, electrodialysates and the duplicate extracts agreed in a range of about 10 per cent.

(5) Addition of atropin to the tissue bath in no way affected the final result of the assay. Hence atropin was not routinely used in most experiments.

(6) Neither the kind of anticoagulant used nor the amount of electrolyte employed in diluting the blood seemed to have any effect on the final results. The oxalate and citrate ions, being anions, were absent from the dialysate to be tested.

(7) In agreement with Barsoum and Gaddum's findings (1935a) no significant changes in the histamine equivalent of the blood occurs on standing. In seven experiments a sample of blood dialyzed immediately was compared with a duplicate sample which had stood for four hours at room temperature before dialyzing. In two experiments the older sample showed about 5 per cent decrease in activity. No changes occurred in samples kept for fourteen hours at ice-box temperature.

Plasma, platelets, and cells: In preliminary studies on the distribution of histamine in blood, inconsistent results were obtained in the determination of the relative histamine equivalents of plasma and formed elements. Observations established the fact that dialysates of plasma frequently gave much higher histamine values than direct application of the plasma to the strip would have led one to expect. Further, plasma from samples centrifuged only a short time contained a greater histamine equivalent than plasma obtained after prolonged centrifuging. Microscopic examination of the briefly centrifuged plasma samples revealed the presence of platelets in large numbers, although the cellular elements were almost entirely absent. This fact led to the following findings:

(1) In two experiments electrodialysates of a mass of platelets obtained by prolonged centrifuging of a platelet-rich, cell-free suspension possessed 65-75 per cent of the activity of a quantity of whole blood equivalent to that from which the platelets had been obtained. The precipitated cellular elements, though washed repeatedly, showed 15-20 per cent of the entire activity, with 5 per cent in the plasma. Although these experiments indicate a large portion of the histamine equivalent to be in the platelets, it was obviously impossible either to recover

all the platelets by this method or to wash to cells free of platelets.

(2) Using the thrombocytocrit technique of measuring platelet volume, we could demonstrate that within narrow limits the ratio of the histamine equivalent of a platelet suspension to the histamine equivalent of the whole blood is the same as the ratio of their corresponding platelet volumes. In five experiments the two ratios agreed to well within 10 per cent. In one experiment the ratio of the histamine equivalents of the platelet suspension to the whole blood was about 15 per cent higher than the ratio of their platelet volumes.

(3) On the basis of eight determinations in five different rabbits we find the platelet volume to have an average value of .85 per cent (range .70-.97 per cent). No correlation could be observed between platelet volume of blood and the total histamine equivalent of blood in different animals.

(4) Application was made of the principle employed in the thrombocytocrit method of Van Allen, namely that if blood, diluted five times with an isotonic electrolyte solution (1.3 per cent sodium oxalate), is allowed to stand, the cellular elements will settle out leaving the platelets in a uniform suspension for several hours in the same concentration as they existed in the blood before sedimentation. In five experiments equal volumes of blood diluted 1:5 with 1.3 per cent sodium oxalate and a platelet suspension obtained by allowing a duplicate portion to sediment for three and one-half hours were dialyzed and the histamine equivalents of the two compared. In three of the five experiments there was no appreciable difference. In two experiments the platelet suspension had a value about 10 per cent higher than that of the whole blood. As was stated above, neither dilution of the whole blood, nor the period of standing has any significant effect on the total histamine equivalent. There is also no increase in the histamine equivalent of plasma during the period of sedimentation.

(5) In four experiments, the histamine equivalent of the sedimented layer of cells, which must necessarily contain the same concentration of platelets as the overlying cell-free suspension, was compared with the histamine equivalent of both the diluted blood and the platelet suspension. Despite the fact that the sedimented layer contained twice the quantity of corpuscular

elements as did the diluted blood, the histamine equivalent in the diluted blood, the platelet suspension, and the sedimented layer of cells agreed in three experiments within the limits of accuracy.

(6) In fifteen experiments the histamine equivalent in plasma was determined and compared with that of whole blood. In most instances the dialysate of the plasma sample was concentrated ten times before assay. The average value for plasma histamine equivalent was 2.8 per cent the histamine equivalent of whole blood.

(7) Electrodialysates of platelet suspensions, plasma, and whole blood, as well as trichloroacetic acid extracts of whole blood, when concentrated by the above procedure and assayed against histamine on the blood pressure of the cat gave values which checked quantitatively to about 10 per cent of those obtained with the guinea pig intestinal strip. The values were the same before and after administration of atropin to the cat.

(8) The histamine activity of electrodialysates of blood was lost after treatment with Norit.

Dog.--Using the trichloroacetic acid extraction procedure and the guinea pig intestinal strip method for assay, we have found that in the dog, contrary to our findings in the rabbit, the platelets are responsible for a relatively small proportion of the total histamine equivalent of the blood in this species.

In two experiments the values for the equivalent concentration of histamine in the blood were $.035 \times 10^{-6}$ and $.025 \times 10^{-6}$. Our results indicate that the ratio of the histamine equivalents of the red cells to plasma is close to 1:1 as was established by Anrep and Barsoum (1935a). In the same two experiments we found that the corresponding histamine values for the platelets were .7% and .5% per cc. of platelets. However since the platelet volume is only about 1 per cent of the whole blood, the platelets would account for only about 15 per cent of the total histamine equivalent of the blood. It is possible that even this value is too high since the platelet suspensions were probably not completely free of cellular elements.

Discussion

There seems but little doubt that the substance dealt

with in the electrodialysates of rabbit blood is histamine. In addition to its pharmacodynamic activities which were found to correspond quantitatively to histamine both in its smooth muscle stimulating action and its depressor activity in the cat, the physico-chemical properties of the active substance in the dialysates resembled those of histamine, namely that it was a basic substance, dialysable through parchment membranes, stable to prolonged boiling in an acid medium, and adsorbed by Norit.

Since the electrodialysates of rabbit blood, on the one hand, and trichloroacetic acid extracts, on the other, agreed quantitatively with regard to their pharmacodynamic properties, it seems safe to assume that in both cases we are dealing with the same substance. Barsoum and Gaddum (1935a) have found such trichloroacetic acid extracts to have no action on the fowl rectal coecum desensitized to histamine.

We have no explanation of the fact that in these studies the histamine values obtained for rabbit blood were one-half to two-thirds lower than the values cited by Barsoum and Gaddum. There is a slight possibility that their figures are in terms of the acid-phosphate of histamine, while ours represent the basic amine, the former having very nearly three times the molecular weight of the latter.

The evidence presented here indicates very strongly that within the limits of accuracy of the methods used, the histamine in the blood of rabbits is located almost exclusively in the platelets, although the possibility of small quantities being present in the cells of the order found in the dog cannot be excluded. Hence Barsoum and Gaddum's statement (1935a), as well as that of Anrep and Barsoum (1935a), that the histamine activity of rabbit blood is largely in the red blood cells is erroneous.

The presence of histamine in the platelets brings to light a rather astounding fact, namely that the histamine equivalent of rabbit platelets per unit weight far exceeds that of any tissue heretofore studied, being well over ten times the value cited for the lung (Thorpe, 1928) which has long been known as the tissue containing more histamine than any other.

Although a final conclusion is hardly justified on the basis of a study of only two species of animals, it seems not unlikely that the remarkable species variation with respect to the histamine content of the blood may rest largely on variations

in the histamine content of the blood platelets in the different species.

Aside from the theoretical interest which the presence of this potent, pharmacologically active substance in the blood platelets may possess, this fact is of significance in the consideration of the toxic properties acquired by blood during the coagulation process. Although it is beyond the scope of this paper to review in any detail the extensive literature on this subject, it might be well to mention a few of the more direct findings and to point out the possible role of histamine in some of these.

Since the earlier investigators of the toxic and pharmacological properties of serum and defibrinated blood employed biological test objects of widely different character as well as testing blood of many species on these preparations, an attempt to correlate their observations would be fruitless. Indeed, later workers have been able to show that many of the properties of shed blood are the resultant of the action of possibly several substances. Some theories as to the chemical nature of one or two of these substances have been advanced.

That freshly defibrinated blood differed in its properties from old defibrinated blood was observed by Freund (1920) who ascribed the properties of the former to a "frühgift" and the latter to a "spätgift." The frühgift was said to cause a vasodilation and a fall in blood pressure, a relaxation of the isolated intestine, and a contraction of the uterus. The spätgift, on the other hand, had a pressor action and stimulated the isolated gut. Freund was able to reproduce the properties of both with a suspension of cat blood platelets which had been damaged by shaking with glass beads.

Later Zipf (1930) came to the conclusion that the frühgift is probably adenylic acid, or one of its derivatives, a view which has received support by Barsoum and Gaddum (1935a). Freund (1935) has recently attributed the spätgift properties of defibrinated blood to tyramine.

Using blood of various species, among others Meyer (1906), Stewart and Zucker (1913), and later Janeway, Richardson and Park (1918), investigated the property of blood serum to contract the isolated arterial strip. The latter workers demonstrated the presence of a substance possessing this property in the blood platelets, and showed it to be dialyzable, heat stable, and

soluble in alcohol.

Heymans, Bouckaert, and Moraes (1932) were able to show that the vasoconstrictor substance in serum was in some respects similar to adrenalin, although this had been denied by some of the earlier workers (O'Conner, 1912, Trendelenberg, 1916).

The depressor action of serum when injected intravenously had long been known. The later work of Freund using a suspension of blood platelets has been referred to above. Phemister and Handy (1927) observed a peripheral vasodilatory action of traumatized blood; this action did not seem to correspond to that of histamine. Investigating the depressor and peripheral vasodilator effects of serum, hemolyzed and whole blood, Feldberg, Flatov and Schilf (1929) compared their action to the combined effects of adrenalin and histamine, but concluded the action was not identical. Zipf (1930) later ascribed the peripheral vasodilator action to adenylic acid; his attempts to discover histamine in the blood failed (1933).

Many investigators have observed that defibrinated blood exerts both a stimulating and a depressing action on the isolated intestine (Cannon and La Paz, 1911, O'Conner, 1912, Stewart and Zucker, 1913, etc.). Working with the rabbit intestine, Dittler (1918) found the intestinal stimulating principal to be dialyzable through parchment and heat stable. The depressing factor was non-dialyzable although Zipf later concluded it to be adenylic acid. Guttentag (1931) extracted blood with alcohol and observed that such extracts caused a contraction of the isolated atropinized guinea pig intestine but had no depressor action in the cat and hence concluded the intestinally-active substance was not histamine. Also using the guinea pig intestinal strip Jürgendsohn (1931) observed a more marked response to defibrinated rabbit blood than to defibrinated blood of the cat. Barsoum and Gaddum (1935a), however, demonstrated that extracts treated with acid and heat to destroy adenylic acid and other interfering substances contained demonstrable amounts of histamine.

No definite conclusions can be reached as to the importance of histamine as a factor in the findings of these various authors. An evaluation of such would involve the consideration of both the histamine content of the blood platelets of the species used as well as the sensitivity of the preparation used to histamine. That intestinally-active substances other

than histamine are present in defibrinated blood, serum, and possibly the platelets is undeniable, since several of these workers observed a contractile action of serum on the isolated rabbit intestine which is quite insensitive to histamine. On the other hand, where histamine-sensitive preparations were used, such as the isolated guinea pig intestine and the isolated arterial strip, histamine was probably a factor. Also, it may have potentiated the depressor and peripheral dilator action of the fröhgift as well as being possibly the main factor in the wheal formation, which Zipf and Wagenfeld (1930) described as also being one of the properties of the fröhgift in defibrinated blood.

We find no support for the distinction raised by one author (Tarras-Wahlberg, 1936) between a free-phase and a bound-phase of the histamine in rabbit plasma. Judging by the high values he cites for the histamine equivalent of plasma, it seems likely that his samples were not completely free of platelets, a view which is borne out by the method he describes for obtaining plasma.

As yet, any postulation regarding the physiological role of histamine in the platelets would be highly conjectural. A theory which might be advanced to account for its relatively high concentration in rabbit platelets is based on the fact that histamine, contrary to its effects in most other species, constricts the arteriols in rabbits (Grant, 1930), hence the liberation of this substance by the breakdown of the blood platelets at the site of injury might be a factor in controlling hemorrhage.

PART III

HISTAMINE IN BLOOD FOLLOWING TRAUMA

The evidence that histamine-like substances may be liberated by the tissues into the blood stream under certain pathological states in quantities sufficient to produce generalized systemic responses seems fairly well established.

That mechanical stimulation of the skin of subjects with Urticaria Factitia may lead to a flushing of the face and increased skin temperature was demonstrated by Lewis and Harmer (1926); an increased gastric acidity similar to that produced by histamine occurred under the same conditions (Kalk, 1929).

More recently a study by Horton et al. (1936) of the local and systemic responses in patients hypersensitive to cold indicated that under the influence of cold, substances gain access to the general circulation producing effects on blood pressure and gastric secretion of a histamine-like character. Attempts to demonstrate such a substance in blood returning from the affected part were without success.

Although the remarkable similarity between the symptom complex accompanying the administration of large doses of histamine and that seen in animals in a state of anaphylactic shock had long been recognized (Dale, 1913), only recently have direct experimental findings given convincing proof that the two are more than superficially alike. Bartosch, Feldberg, and Nagel (1932) demonstrated the liberation of a histamine-like substance from the lungs of sensitized guinea pigs when administered a shocking dose of the antigen. Shortly later a similar finding was reported for dogs by Gebauer-Fuelnegg, Dragstedt, and Mullenix (1932) who demonstrated the presence of a substance in the thoracic duct lymph of the dog in anaphylactic shock possessing both the pharmacological and chemical properties of histamine.

There has been evidence advanced that under physiological conditions as well as in pathological states histamine may be liberated into the blood stream. Barsoun and Gaddum (1935b) first

showed an increase in the histamine content of venous blood from an extremity to which the circulation had been occluded for varying lengths of time. Later Anrep and Barsoum (1935b) found that muscular contraction is accompanied by a marked increase in the histamine concentration of the venous blood.

However, the relation of histamine to the condition of circulatory collapse which follows massive destruction of tissues is a matter of controversy. Although Cannon and Bayliss (1919), in reporting experiments performed chiefly on cats, came to the conclusion that a toxic factor plays the principal role in bringing about the oligemia and extreme hypotension which characterize traumatic shock, their evidence was largely indirect. They had demonstrated that following severe trauma to the denervated extremity of the experimental animal, the blood pressure would sink to shock level within the course of an hour. If, however, the vessels to the leg were tied, this fall in pressure did not occur.

This evidence supported by the findings of Dale and Laidlaw (1919), that small quantities of histamine injected into the circulation could bring about a profound fall in pressure resembling shock, prompted Cannon to formulate the theory of traumatic toxemia which ascribed the loss in blood volume and fall in pressure following extensive tissue damage to the absorption of protein split products of histamine-like character from the area of trauma.

Although this theory enjoyed almost universal acceptance for a number of years following the war, serious criticisms were first raised by M. I. Smith (1928), who, being unable to demonstrate depressor substances in blood from an injured extremity, first suggested that local fluid loss into the injured area rather than the absorption of a circulatory poison was probably the more important factor in causing the oligemia.

Since then numerous investigations have tended to support this view (Parsons and Phemister, 1930; Blalock, 1930; Freedlander and Lenhart, 1932; Holt and MacDonald, 1934). None of these workers were able to detect the presence of a depressor substance resembling histamine in blood returning from the traumatized area. Blalock (1930) noted that the increase in weight of the traumatized extremity indicated a loss of blood and plasma equivalent to almost half the blood volume, which in itself could account for

the fall in pressure. He pointed out that Cannon and Bayliss by their method of amputation had not obtained the true weight increase in the traumatized limb.

In addition to the evidence that local fluid loss may be the initiating factor in producing the fall in pressure, other investigators have determined that the circulatory changes follow a different sequence after muscle trauma than in histamine poisoning (Johnson and Blalock, 1931; Burch and Harrison, 1930).

Roome, Keith, and Phemister (1932), after producing a permanently low blood pressure in a variety of ways, studied the relative effects in these different cases of removing small quantities of blood. It was observed that in those cases in which low pressure had been produced by muscle trauma or hemorrhage, removal of small quantities of blood was fatal while animals in histamine shock were resistant to the loss of much larger quantities.

By establishing a semipermeable barrier between the circulations of two animals, O'Shaughnessy and Slome (1935) were able to produce a fall in pressure in one animal upon injecting histamine into the other. However, no fall in pressure occurred in the first animal when the extremity of the second had been subjected to trauma sufficient to produce shock.

Using the isolated intestinal strip and cat blood pressure as biological detectors of histamine, Dragstedt and Mead (1937) tested heparinized dog plasma before and after shock had been induced by muscle trauma and intestinal manipulation. Their results were negative, although they state their preparations could detect histamine if present in a concentration of 4×10^{-6} . Histamine injected intramuscularly in quantities sufficient to produce shock could be demonstrated in the blood plasma by these methods.

In reviewing the literature it has been noted that by and large the investigators have sought only to determine whether or not a histamine-like substance is the primary etiologic factor in shock. It seems fairly well established that in experimental shock of the type generally studied, namely that produced by massive trauma to an extremity, local fluid loss is the initiating and perhaps the most important factor in bringing about the oligemia and hypotension. Nevertheless it cannot be said on the evidence thus far presented that a toxic factor does not enter in.

It may be added that most of the authors mentioned have either expressed or implied that such a reservation be made.

In view of the recent developments in extraction procedure

TABLE III
HISTAMINE IN BLOOD AFTER TRAUMA

Dog Number	Before trauma	After trauma		% Diff
		Normal leg	Traumatized leg	
	γ per cc.	γ per cc.	γ per cc.	
1	.025	.020	.040	100
2	.020	.020	.045	125
3	.013	.015	.020	33
4	.025	.025	.045	80
		.027	.040	48
5	.017	.017	.030	73
		.015	.027	80
6	.018	.018	.035	94
		20 min.	.028	55
		40 min.	.022	22

through the application of which the presence of histamine in normal blood has been well established (Barsoum and Gaddum, 1935a), we undertook to reinvestigate this problem using the direct method of estimating quantitatively the histamine concentration in normal blood as compared with that in blood returning from a traumatized area.

Experimental Procedure

Dogs anaesthetized with sodium barbital, .3 gm/kg administered intraperitoneally, were used in all experiments.

Blood pressure was recorded from the cannulated carotid artery with a mercury manometer, records being taken one minute in every ten during the course of the experiment.

In two animals both sciatics and both femoral nerves were sectioned during the control period.

The femoral sheath in each leg was exposed; in the four later experiments, a small, heavy walled rubber tubing was passed beneath each sheath and its contents. This could be tightened around the proximal portion of the thigh to act as a tourniquet when required. A control record having been taken, the flexor group of muscles in one thigh was subjected to fifty blows with a heavy wooden mallet, this being repeated at intervals of thirty to forty minutes until shock level in pressure had been produced.

Blood samples were collected with a 20 gauge needle and syringe containing .5 cc. 5 per cent sodium citrate. Eleven cc. were taken for each sample, the citrate and blood mixed in the syringe, 1.5 cc. removed for hematocrit determinations and the remainder expelled into a 50 cc. centrifuge tube containing 15 cc. 10 per cent trichloroacetic acid.

In every experiment two samples were obtained during the control period, one from each femoral vein. In two experiments a lifting ligature being placed around the vein, samples were removed distally to the ligature. In four experiments the tourniquet around the thigh of the leg to be traumatized was tightened, a clamp then being placed on both artery and vein. Immediately following the trauma, the clamp was removed from the artery and a sample taken from the vein distal to the clamp. Excepting the trauma the same procedure was then followed in obtaining the sample from the normal leg.

In three experiments samples were obtained from each femoral vein only after the first period of trauma, in two experiments after the first and second periods of trauma, and in one experiment three samples were collected from the traumatized leg, one immediately following trauma and the other two twenty minutes and forty minutes thereafter.

In two experiments arterial samples were also obtained, in each case one before trauma, the other when the animal was in a profound shock.

In one control experiment arterial and venous samples were collected as above but no trauma was employed. To two of these samples, one gamma of histamine was added.

The method of extraction followed was identical to that described by Barsoum and Gaddum. In our experience 85 to 90 per

cent of the histamine added to samples before extraction could be recovered.

The final aqueous extracts were assayed against a histamine standard ($.1 \times 10^{-6}$) using the atropinized guinea pig intestinal strip procedure described in Part II of this paper. In these experiments an accuracy of about ± 5 per cent was obtained.

Results

In every experiment the sample of venous blood obtained from the femoral vein of the traumatized leg gave a higher histamine equivalent concentration than did venous blood from the normal leg. The average increase was 79.5 per cent (see table).

There was no significant difference in the histamine equivalent concentration in venous blood obtained before and after trauma from the normal leg.

In two experiments the histamine equivalent concentrations of arterial blood samples obtained before trauma and after the animal was in a state of deep shock were practically identical.

In one experiment, the sample obtained immediately after trauma gave a value 94 per cent greater than the normal, this dropping to 55 per cent in twenty minutes, and after forty minutes had reached a level still 22 per cent higher than the normal.

In the control experiment, three samples of venous blood and two of arterial blood were all found to have values within a range of ± 6 per cent.

Discussion

In this study we have attempted to duplicate, as far as possible, the experimental conditions employed by other workers who have investigated the etiology of experimental shock. Our principal aim has been to determine, by objective methods, whether, under the conditions of the experiment, substances of a histamine-like character increase in blood returning from a traumatized limb.

That the active substance in the extracts prepared of the blood was probably histamine itself has been demonstrated by Barsoum and Gaddum. Although histamine is but one of five substances present in blood extracts which have an effect on the

isolated guinea pig intestine, two of the others, namely adenosine and the "spätgift" are destroyed by the acid treatment. The effects of choline and its principal ester, acetylcholine, are ruled out by paralyzing the strip to their action with atropin. Hence histamine is the only remaining substance present in the extracts which acts on the smooth muscle of the intestine. It would have been well to have confirmed our results on the fowl rectal coecum before and after histamine desensitization. This, however, has not been done.

Although the histamine concentration of the venous blood from the traumatized extremity was greater than that of the control leg, the fact that no appreciable change occurred in the histamine level of either arterial or normal venous blood after trauma raises doubt as to whether the histamine added by the injured tissue exerted any significant effect on the systemic circulation. It is possible that the lungs play a role in removing excess histamine from the venous blood.

In view of the work of Anrep and Barsoum (1935b) who observed marked increases in the histamine concentration of venous blood during muscular contraction and that of Barsoum and Gaddum (1935b) who demonstrated an increase in venous blood histamine after occlusion of the circulation to a limb, it seems likely that both of these factors may play a role in some of our findings following trauma. However, in two experiments, in which the extremities were denervated, the factor of muscular activity was probably ruled out.

In conclusion it may be said that there is a distinct increase in the concentration of histamine-like substances in blood returning from a traumatized limb as compared with blood obtained from a normal extremity. However, whether the increase is significant as a factor in traumatic shock and whether other toxic factors may also be involved are problems awaiting further investigation.

PART IV
GENERAL SUMMARY AND CONCLUSIONS

Part I

1. Quantitative studies have been made on the histamine-like substances in alcoholic extracts of the skin of the albino rabbit following ultra-violet irradiation and burns.
2. The substances studied resembled histamine
 - (a) in producing a depressor response in the etherized vagotomized cat with or without the administration of atropin
 - (b) in having no effect on the blood pressure of the rabbit under deep ether anaesthesia
 - (c) in being completely adsorbed by Norit
3. Following irradiation an increase in these depressor substances was observed but only if erythema had appeared.
4. Following burns there was a decrease in the histamine content of the skin if the skin was removed several hours after burning.
5. The Pauly reaction of extracts of irradiated skin was distinctly increased as compared with the controls. The increase was less marked in extracts of burned tissue.
6. Attempts to demonstrate consistent changes in the histamine content of venous blood from burned or irradiated areas were unsuccessful.
7. It is concluded that burns and irradiation have opposite effects on the histamine-content of the skin and therefore that Lewis' theory of the liberation of preformed histamine following injury probably does not hold for injury by ultra-violet irradiation.

Part II

1. A modified method of histamine assay using the isolated guinea pig intestinal strip is described, whereby the use of preserved strips and the addition of excess calcium to the tissue bath have increased the reliability and accuracy of the method.

2. Quantitative studies have been made on the presence and distribution of histamine-like substances in the blood of the rabbit and dog. The histamine equivalents of electro-dialysates and trichloroacetic acid extracts agree quantitatively on two different biological assay objects, thus giving added support to the view that the substance is histamine.
3. Contrary to the statements of other authors, the histamine-like activity of rabbit blood is located almost exclusively in the platelets. In dog blood, on the other hand, the platelets possess only a small share of the total blood histamine.
4. The possible significance of histamine as one of the toxic factors in serum and defibrinated blood is discussed.

Part III

1. Using the procedure of Barsoum and Gaddum we have investigated the concentration of histamine-like substances in the blood of the dog before and after trauma to an extremity.
2. In six experiments the average concentration of these substances in venous blood from the traumatized leg was 80 per cent higher than in blood from the normal leg.
3. No appreciable difference could be observed in the concentration of histamine-like substances of arterial blood before trauma and when the animal was in a deep state of shock.
4. The possible significance of these findings in obtaining a more complete picture of the etiologic factors in traumatic shock awaits further investigation.

LIST OF REFERENCES

- Ackermann, D., and Kutscher, F. 1910. Die physiologische Wirkung einer Secalebase und des Imidazolyläthylamins. Ztschr. f. Biol., 65: 504.
- Anrep, G. V., and Barsoum, G. S. 1935a. Distribution of histamine between plasma and red blood corpuscles. J. Physiol., 85: 36P.
- Anrep, G. V., and Barsoum, G. S. 1935b. Appearance of histamine in the venous blood during muscular contraction. J. Physiol., 85: 409.
- Barger, G., and Dale, H. H. 1910. Beta-Iminazolylethylamine and the other active principles of ergot. Proc. Chem. Soc., 26: 128.
- Barger, G., and Dale, H. H. 1911. Beta-Iminazolylethylamine, a depressor constituent of intestinal mucosa. J. Physiol., 41: 499.
- Barsoum, G. S., and Gaddum, J. H. 1935a. The pharmacological estimation of adenosine and histamine in blood. J. Physiol., 85: 1.
- Barsoum, G. S., and Gaddum, J. H. 1935b. The liberation of histamine during reactive hyperemia. J. Physiol., 85: 13P.
- Bartosch, R. 1936. Über die Freisetzung von Histamin durch chemisch bekannte Substanzen. Arch. f. exper. Path. u. Pharmacol., 181: 176.
- Bartosch, R., Feldberg, W., and Nagel, E. 1932. Die Freiwerden eines histaminähnlichen Stoffes bei der Anaphylaxie des Meerschweinchens. Arch. f. d. ges. Physiol., 230: 129, 674.
- Best, C. H., et al. 1927. The nature of the vaso-dilator constituents of certain tissue extracts. J. Physiol., 62: 397.
- Blalock, A. 1930. Experimental shock: cause of low blood pressure produced by muscle injury. Arch. Surg., 20: 959.
- Burch, J. C., and Harrison, T. R. 1930. The effect of spinal anaesthesia on cardiac output. Arch. Surg., 21: 330.
- Burn, J. H. 1928. Methods of Biological Assay. Oxford University Press.
- Cannon, W. B., and Bayliss, W. M. 1919. Traumatic toxemia as a factor in shock. Med. Res. Comm. Spec. Rep. 26.

- Cannon, W. B., and De La Paz. 1911. Emotional stimulation of adrenal secretion. *Am. J. Physiol.*, 28: 64.
- Dale, H. H. 1913. The anaphylactic action of plain muscle in the guinea pig. *J. Pharmacol.*, 4: 167.
- Dale, H. H., and Laidlaw, P. P. 1910. The physiological action of Beta-aminazolyethylamine. *J. Physiol.*, 41: 318.
- Dale, H. H., and Laidlaw, P. P. 1919. Histamine shock. *J. Physiol.*, 52: 355.
- Diehl, F. 1931. Die Wirkung der Ultraviolettbestrahlung der Haut. *Arch. f. exper. Path. u. Pharmacol.*, 159: 361.
- Dittler, R. 1918. Über die Wirkung des Blutes auf den isolierten Dünndarm. II. Mitteilung. *Ztschr. f. Biol.*, 68: 223.
- Dragstedt, C. A., and Mead, F. B. 1937. A pharmacologic study of the toxemia theory of surgical shock. *J. Am. Med. Ass'n*, 108: 95.
- Ebbecke, U. 1922. Über elektrische Hautreizung. *Arch. f. d. ges. Physiol.*, 195: 300.
- Ellinger, F. 1928. Über die Entstehung eines den Blutdruck senkenden und den Darm erregenden Stoffes aus Histidin durch Ultraviolettstrahlung. *Arch. f. exper. Path. u. Pharmacol.*, 136: 129.
- Feldberg, W., Flatow, E., and Schilf, E. 1929. Die Wirkung von Blut und Serum auf Warmblütergefäße. *Arch. f. exper. Path. u. Pharmacol.*, 140: 129.
- Feldberg, W., and Schilf, E. 1930. *Histamin*. Berlin: Springer.
- Freund, H. 1920. Über die pharmakologischen Wirkungen des defibrinierten Blutes. *Arch. f. exper. Path. u. Pharmacol.*, 86: 266; 88: 39.
- Freund, H. 1935. Über Tyraminwirkung und Spätgift. *Arch. f. exper. Path. u. Pharmacol.*, 180: 189.
- Freedlander, S. O., and Lenhart, C. H. 1932. Traumatic shock. *Arch. Surg.*, 25: 693.
- Gaddum, J. H. 1936. Gefässerweiternde Stoffe der Gewebe. Leipzig: Georg Thieme.
- Gebauer-Fuelnegg, E., Dragstedt, C. A., and Mullenix, R. B. 1932. A physiologically active substance appearing during anaphylactic shock. *Proc. Soc. exp. Biol.*, 29: 1084.
- Guggenheim, M., and Loeffler, W. 1916. Biologischer Nachweis proteinogener Amine in Organextrakten und Körperflüssigkeiten. *Biochem. Ztschr.*, 72: 303.
- Guttentag, O. E. 1931. Histamin und histaminartige Substanzen im Blut. *Arch. f. exper. Path. u. Pharmacol.*, 162: 727.

- Hanke, M. T., and Koessler, K. K. 1920. The quantitative colorimetric estimation of histamine in protein and protein-containing matter. *J. Biol. Chem.*, 43: 527, 543.
- Hanke, M. T., and Koessler, K. K. 1924. XX. On the presence of histamin in the mammalian organism. *J. Biol. Chem.*, 59: 879.
- Harpuder, K. 1935. Production of vasodilating substances (histamine and acetylcholine) in the skin by physical therapy. *Arch. Phys. Therap.*, 16: 23.
- Harris, K. E. 1927. Observations upon a histamine-like substance in skin extracts. *Heart*, 14: 161.
- Heymans, C., Bouckaert, J. J., and Moraes, A. 1932. Inversion par l'ergotamine de l'action constrictive des "vasotonines" du sang défibriné. *Arch. internat. de pharmacodyn. et de therap.*, 43: 468.
- Holt, R. L., and MacDonald, A. D. 1934. Observations on experimental shock. *Brit. Med. Journ.*, (1): 1070.
- Horton, B. T., Brown, G. E., and Roth, G. M. 1936. Hypersensitivity to cold with local and systemic manifestations of a histamine-like character. *J. Am. Med. Ass'n*, 107: 1263.
- Janeway, T. C., Richardson, H. B., and Park, E. A. 1918. Experiments on the vasoconstrictor action of blood serum. *Arch. Int. Med.*, 21: 565.
- Johnson, G. S., and Blalock, A. 1931. Experimental shock. *Arch. Surg.*, 23: 855.
- Jürgendsohn, E. 1931. Die Wirkung von frischem und defibriniertem Blut auf den Meerschweinchendünndarm. *Arch. f. exper. Path. u. Pharmacol.*, 162: 739.
- Kalk, H. 1929. Zur Frage der Existenz einer histaminähnlichen Substanz beim Zustandkommen des Dermographismus. *Klin. Wschr.*, 8: 64.
- Krogh, A. 1929. *Anatomy and Physiology of Capillaries*. 2nd edit., New Haven.
- Lewis, T. 1927. *The Blood Vessels of the Human Skin and Their Responses*. London.
- Lewis, T., and Harmer, I. M. 1926. Release of vaso-dilator substances in injuries of the skin. *J. Physiol.*, 62: 11P.
- Loos, H. O. 1931. Über die Beziehungen des Histamins zur Entzündung. *Arch. Dermatol. u. Syph.*, 164: 199.
- MacGregor, R. G., and Thorpe, W. V. 1933. The extraction of histamine from tissues by electro-dialysis. *J. Physiol.*, 77: 33P.
- MacGregor, R. G., and Thorpe, W. V. 1933. The quantitative extraction of histamine from tissues by electro-dialysis. *Biochem. J.*, 27: 1394.

- Major, R. H., and Weber, C. J. 1929. Observations on the depressor substances in certain tissue extracts. *J. Pharmacol.*, 37: 367.
- Menkin, V. 1936. Studies on inflammation. XII. Mechanism of increased capillary permeability. A critique of the histamine hypothesis. *J. Exper. Med.*, 64: 485.
- Meyer, O. B. 1906. Über einige Eigenschaften der Gefäßmuskulatur. *Ztschr. f. Biol.*, 48: 352.
- O'Conner, J. M. 1912. Über den Adrenalingehalt des Blutes. *Arch. f. exper. Path. u. Pharmacol.*, 67: 95.
- O'Shaughnessy, L., and Slome, D. 1935. Etiology of traumatic shock. *Brit. Journ. Surg.*, 22: 589.
- Page, I. H. 1935. Observations on the depressor extracts of human blood and on the vascular action of extracts of rabbit and dog blood. *J. Exper. Med.*, 61: 97.
- Parsons, E., and Phemister, D. B. 1930. Hemorrhage and "shock" in traumatized limbs. *Surg. Gyn. and Obstet.*, 51: 196.
- Phemister, D. B., and Handy, J. 1927. Vascular properties of traumatized and laked bloods. *J. Physiol.*, 64: 155.
- Popielski, L. 1909. Über die physiologische Wirkung von Extracten aus sämtlichen teilen des Verdauungskanales. *Arch. f. d. ges. Physiol.*, 128: 191.
- Roome, N. W., Keith, W. S., and Phemister, D. B. 1933. Experimental shock: The effect of bleeding after reduction of blood pressure by various methods. *Surg. Gyn. and Obstet.*, 56: 161.
- Smith, M. I. 1928. Studies on experimental shock with special reference to its treatment. *J. Pharmacol.*, 32: 465.
- Stewart, G. N., and Zucker, T. F. 1913. A comparison of the action of plasma and serum on certain objects used in biological tests for epinephrin. *J. Exper. Med.*, 17: 152.
- Tarras-Wahlberg, B. 1936. On the presence and appearance of histamine in blood and lungs. *Skand. Arch. f. Physiol.*, 73: Supplement.
- Thorpe, W. V. 1928. Vasodilator constituents of tissue extracts. *Biochem. J.*, 22: 94.
- Trendelenberg, P. 1916. Über die Adrenalin Konzentration im normalen Blut. *Arch. f. exper. Path. u. Pharmacol.*, 79: 154.
- Van Allen, C. M. 1926. Volume measurement of blood platelets. *J. Lab. Clin. Med.*, 12: 282.
- Windaus, A., and Vogt, W. 1907. Synthese des Imidazolyläthylamins. *Ber. d. Deut. Chem. Gesellschaft*, 40: 3691.

- Zipf, K. 1930. Die chemische Natur des Frühgiftes. Arch. f. exper. Path. u. Pharmacol., 157: 97.
- Zipf, K., and Hülsmeier, P. 1933. Histaminähnliche Stoffe und Spätgift im Blut. Arch. f. exper. Path. u. Pharmacol., 173: 1.
- Zipf, K., and Wagenfeld, E. 1930. Über die pharmakologische Wirkung des frisch defibrinierten Blutes. Arch. f. exper. Path. u. Pharmacol., 150: 70.

